NMR studies on the structure and function relationships of proteins by using artificial structural perturbations

(構造摂動を利用した NMR によるタンパク質の構造機能相関研究)

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ABSTRACT

Artificial perturbation to protein structure discriminates the functional roles of structure from dynamics. Protein structure is maintained under limited thermodynamic energy. Even small structural modifications including single amino acid change or chemical modification to the limited residues in protein, therefore, perturb its structure and dynamics to modulate its function. NMR can decipher protein structure and dynamics at atomic level. In my Ph.D. research, I intended to grasp the details in the structure/dynamics-function relationships by extensively use of NMR to the artificially modified proteins.

I focused on two different types of proteins. One is the prolyl-cis/trans-isomerase (PPIase) that constitutes important reactions in the phosphor-mediated signal transduction pathways in cells. The other is a DNA binding protein HMGB1 that is also known to work as an 'alarmin' to let cells know that something lethal reactions are happening in the neighboring cells. In either case, the structural perturbation in combination with various types of NMR analyses gave more profound structure/dynamics-functional relationships that were not ever gained.

Allosteric breakage to the hydrogen bond within the dual-histidine motif in the active site human Pin1 PPIase (chapter II)

Pin1 PPIase is an essential enzyme that specifically catalyzes the phosphorylated-Ser/Thr-Pro motifs. The residues C113-H59-H157-T152 form a hydrogen bond network in the active site. A study from computer simulation has shown that protonation of C113 rearranges the hydrogen bonding network, meanwhile, changing the tautomeric states of adjacent histidines (H59 and H157). We employed two Pin1 PPIase mutants to mimic the protonation state of C113 to see how the change in C113 modulates the active structure, dynamics and function. In the current

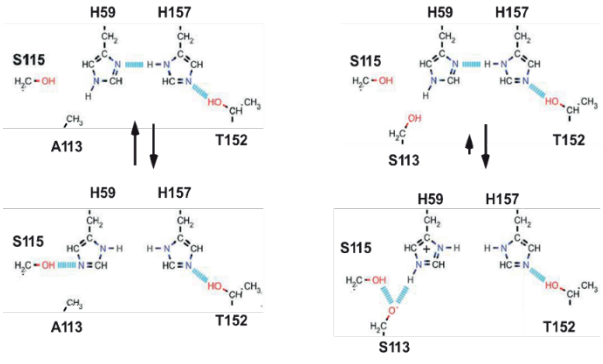

Fig. 1. Dynamics in the hydrogen bond network in C113 mutants.

studies, we found the C113A and C113S Pin1 mutants altered the protonation states of H59, which change the active site structure by changing hydrogen bonding network running in the active site (Fig.1). The present results experimentally demonstrated that the residue change at 113 site can modulate the dynamics in the hydrogen bond network. This ensures C113 can role as a pivot to drive the concerted function among the residues in the hydrogen bond network as theoretically predicted.

Redox-sensitive structural change in the A-domain of HMGB1 and its implication for the cisplatin modified DNA (chapter III)

HMGB1 (high-mobility group B1) is a ubiquitously expressed bifunctional protein. HMGB1 changes its functions according to the redox states, in both intra- and extra-cellular environments. Two cysteines, Cys23 and Cys45, in the A-domain of HMGB1 forms a disulfide bond under oxidative conditions. The structure of the oxidized A-domain in HMGB1 has not been solved, therefore, the molecular mechanism for the functional changes in reposed to the redox states of HMGB1 remains elusive. We solved the oxidized domain structure and compared with the reduced one. The flipped phenyl ring at Phe38 in the oxidized A-domain has demonstrated that the phenyl ring could not readily intercalate into the cavity by the guanine bases (Fig.2), which could explain the reduced affinity of the oxidized HMGB1 to the cisplatinated DNA. The conformational change was unexpected from the high-resolution structure under reductive condition as inside cells. This gives an example for showing nature uses the naturally occurring chemical modification to modulate protein function through changing the structure.

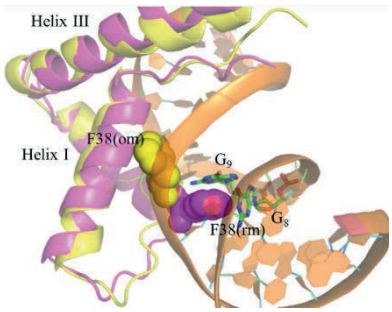

Fig.2. Structure comparison between oxidized complex (yellow) and reduced (magenta)

Conclusion

Protein structure/dynamics-function relationships on two types of proteins were explored in my Ph.D. research. In either case, I uncovered that the very subtle modification to protein structure significantly changed the functionally relevant structure and/or dynamics. I want to emphasize that the results in such details could not be gained without a variety of NMR analyses I applied. Expansion of the use of NMR to proceed our understanding on the protein structure/dynamics-function relationships was also aimed in my research, and it was achieved.